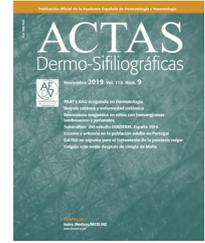




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REVIEW

Update on the Diagnosis of Sexually Transmitted Infections[☆]



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Abstract Sexually transmitted infections (STIs) are one of the most frequent and universal Public Health problems. Health professionals should be aware of the possibility of STIs due to their high morbidity and the presence of sequelae. The delay in the diagnosis is one of the factors that justifies the difficulty to infections control. Diagnostic tests allow the introduction of aetiological treatment and also leads to treating symptomatic and asymptomatic patients more effectively, as well as to interrupt the epidemiological transmission chain without delay. In this review we have made an update of the main existing diagnostic methods for the more important STIs.

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Actualización en el diagnóstico de las infecciones de transmisión sexual

Resumen Las infecciones de transmisión sexual (ITS) son uno de los problemas de salud pública más frecuentes y universales. Debido a que las ITS son responsables de una alta morbilidad, así como de secuelas graves, es muy importante que todos los profesionales de la salud las

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Prueba de
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tengan en cuenta en el momento de valorar al paciente. La dificultad en el control de las ITS se debe principalmente al retraso diagnóstico. Las pruebas diagnósticas permiten realizar un manejo etiológico, así como facilitar un tratamiento más efectivo tanto de los pacientes sintomáticos como de los asintomáticos, y finalmente permitirán interrumpir de una forma más precoz la cadena epidemiológica de transmisión. En la presente revisión, se ha llevado a cabo una actualización acerca de los principales métodos diagnósticos existentes en las ITS más relevantes.

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Introduction

Sexually transmitted infections (STIs) are a globally increasing public health problem with an estimated one million new infections each day. Though largely preventable, they have a wide range of negative consequences on individual health, ranging from acute conditions to severe complications, long term sequelae and also increase the risk for HIV acquisition. Beyond the significant morbidity and mortality, STIs also represent an enormous economic burden.

Quantifying STI prevalence and incidence remains a critical component of global monitoring for planning interventions, treatment recommendations and advocating for resources.¹ Most countries conduct universal case reporting and some of them also use sentinel sites. However, there is a general lack of standardization among countries and many have yet to implement STI surveillance systems.

Each year, there are estimated 357 million new cases of four curable STIs among people aged 15-49 years: *Chlamydia trachomatis* is acquired by 131 million persons, 78 million get *Neisseria gonorrhoeae*, 5.6 million syphilis and 143 million contact trichomoniasis worldwide. The prevalence of some viral sexually STI is similarly high. More than 500 million people are living with virus herpes simplex type 2 infection. However, the epidemiology of HSV is changing and genital herpes is increasingly being caused by HSV-1 in industrialized countries. Human papillomavirus (HPV) infection is the most prevalent sexually transmitted infection in men and women worldwide. More than 290 million women have an human papillomavirus (HPV) infection. The prevalence varies by region and gender.² Genital warts represent a significant public health problem. The European Centers for Disease Prevention and Control (ECDC) surveillance report on STI in Europe and Red Nacional de Vigilancia Epidemiológica (RENAVE) in Spain shows the epidemiological features and basic trends of the five STI under European Union (EU) surveillance: *Chlamydia trachomatis* infection, gonorrhoea, syphilis, congenital syphilis, and Lymphogranuloma venereum (LGV).^{3,4}

Chlamydia was the most common STI in Europe with 394.163 cases reported in 2015. In Spain, 7.162 new infections were notified in 2016. The incidence rate was estimated to be 18 per 100,000 (with values ranging among

different regions, the highest was 46,4 in Cataluña). How 53% of cases reported were in females. The majority of reported cases continue to be among young people between 15 and 24 years of age, suggesting that testing continues to be targeted towards groups at higher behavioural risk of STI while simultaneously aiming to reduce the risks of reproductive tract complications.^{4,5} But while trends in the number of chlamydia infections appear to have stabilised in recent years (2011-2015, increased by 4% overall), gonorrhoea rates have gone up by 79% since 2008, particularly among men. With more than 75.000 reported cases in 2016, gonorrhoea is the second most commonly notified STI in Europe, and Spain is not an exception with 6.456 cases reported, 83% in men. Male to female ratio in Spain was 5:1. This increase seems to be linked to increased case numbers among men who have sex with men (MSM).^{4,6} The number and rate of reported syphilis cases has continued to increase in 2015 (28 701 syphilis cases in Europe). The increases continue to be driven by cases reported among men, specifically among MSM (62%). Trends among heterosexual men and women, on the other hand, appear stable or show a slight decrease. Syphilis rates increased in Spain until 2011, but there has been an stabilization since then with 3886 cases notified in 2015 and 3357 in 2016.^{4,7} Following a decreasing trend, the notification rate of congenital syphilis has stabilised since 2006. 42 cases were reported in Europe in 2015 and four cases in Spain in 2016.^{4,8}

Finally, the number of reported cases of LGV continued to increase in western and central European countries with 1787 in 2015. Spain notified 248 cases in 2016. Epidemiological investigations suggest that transmission is mainly among HIV-positive MSM engaging in high-risk practices.^{4,9} Infection with the human immunodeficiency virus (HIV) and STIs are clearly interrelated, sharing risks, incidence and transmission mechanisms. Currently, the global rate of new HIV diagnoses in Spain is at levels similar to those of other countries in the WHO European Region. Since 2003, a record of new diagnoses of HIV infection in our country has been made, with a stable number in the last 7 years, standing at an average of 3293 cases/year.

This review highlights the main diagnostic methods currently used for STIs community to ensure that effective interventions for STIs prevention, screening, diagnosis, and treatment are made more widely available.

Chlamydia trachomatis infections

Several factors may account for the increase of diagnosed CT infections, including changes in sexual behaviour and lack of prevention and education, but also more frequent testing with improved detection systems. Testing for Chlamydia is indicated in patients with urogenital, cervicitis, pelvic inflammatory disease (PID), and extragenital infection by sexual transmission: anorectal, pharyngeal and ocular.¹⁰ Initially, all relevant clinical materials can be analyzed by molecular biology techniques. Non invasive specimens are the preferred materials, in particular for screening of asymptomatic persons. First void urine and urethral swabs from male patients are equivalent regarding to performance of nucleic acid amplification test (NAATs). Collection of urine sample is much better accepted and therefore the recommended sample in men.^{11,12} To detect extra-genital CT infections, testing of corresponding swabs or tissue samples is required. CT infection of men who have sex with men (MSM) is frequently localized in the rectum or pharynx, without causing any symptoms, and it require testing of appropriate oral and anal swabs to be diagnosed. In this case, the only screening of an urine sample can lead to a infradiagnostic of the infection.^{13,14}

Diagnostic Chlamydia trachomatis (CT) infections

Nucleic Acid Amplification Test (NAATs)

Only if *C. trachomatis* NAATs are not available or affordable, isolation of *C. trachomatis* in cell culture or identification of *C. trachomatis* by direct fluorescence assays (DFA) can be used for diagnosis of acute infections. Validated and quality assured NAATs are recommended due to their superior sensitivity, specificity and speed of diagnosis of both symptomatic and asymptomatic chlamydial infections compared to all other diagnostic techniques.^{15,16} Due to the high specificity of the appropriately validated *C. trachomatis* NAATs and risk of losing low positive result in repeated testing, confirmatory testing of positive specimens is not recommended.^{17,18} An important exception is represented by legal investigations in case of sexual assault.

The NAATs can use less invasively collected specimens such as urine samples in men or vulvo-vaginal swabs in women and anorectal swabs in both genders.¹⁹ Diagnostic sensitivity can be increased using coated magnetic beads nucleic acids nucleic acids were isolated in higher quantity and quality.²⁰ These bead based extraction systems can be automated and use in several high-throughput systems that allow simultaneous testing of chlamydia and gonococci with sensitivity and specificity.²⁰ In order to increase the sensitivity of the technique, these tests are based on the detection of genes present in high number of copies (cryptic plasmid X06707 (10 copies /genome) or 16S ADNr (2 copies/genome)).²¹

NAATs have detected 10-30% more *C. trachomatis* positive specimens than culture in studies comparing the two methods.^{22,23} In some several studies, results of differ-

ent NAATs were show to be highly concordant.^{24,25} The importance of genetic variation became evident with the appearance of the Swedish variant that was not detected by some commercial NAATs due to a deletion in the target region of these test.²⁶ The implementation of a 2nd target region in NAATs represents an important improvement of NAATs, allowing detection of new variants with deletions or recombination in one of the target region.²⁷ As an example of systems that developed a modification of the technique Cobas Taqman CT/NG v 2.0 (Roche) and Artus *C. trachomatis* plus RG Kit PCR (Qiagen). One limitation of all these diagnostic techniques is the lack of discrimination between the different biovars of *C. trachomatis* related to different pathological processes that can be detected in urethral or cervical samples. None of the above technique can discriminate between serovars D-K and serovars L1-L3 related with lymphogranuloma venereum (LGV).²¹

Point of care test (POCTs)

Rapid point-of-care test provide a quick and easy test result, and diagnosis and subsequent treatment can be provided at the same visit at clinic or even in remote setting. Most POCTs are immune chromatographic test based on lateral flow technology and detect chlamydia lipopolysaccharide antigen (LPS) in genital swabs or urine. Compared with culture and NAATs, these antigen based in POCTs are significantly less sensitive and less specific. The antigen based POCTs were not recommended for *C. trachomatis* testing of both asymptomatic screening and symptomatic patients.²⁸ POCTs with increased sensitivity have been developed and new POC NAATs (e.g. Xpert assay of Cepheid CT/NG). This assay is based on real time PCR carried out in a closed system. After application of the clinical sample to a cartridge, the subsequent steps of nucleic acid isolation, amplification and detection of PCR products proceed in a fully automated process. Another commercial test POCTs NAATs use technology isothermal amplification, like loop mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA).²⁹

Serology

Testing for *C. trachomatis* antibodies is not useful to diagnose local epithelial infectious of the lower genital tract, because antibodies are detectable with a delay of several weeks, antibody titers may be low, and many serologic test are not able to differentiate antibodies against different chlamydia species.

The microimmunofluorescence (MIF) test was long time considered the reference method of chlamydia antibody testing but enzyme immunoassays (EIA) and immunoblots or line assays are currently used more frequently to detect chlamydia infections.^{30,31} The EIA technology is based on the detection of antigen by measuring a colored signal generated by the antigen reaction liposaccharides (LPS) with the antibody. Traditionally they have enjoyed great popularity for being simple, objective and automated techniques. The specificity of EIA is low, being able to give false positives due to the presence of bacterial lipopolysaccha-

rides LPS. Other techniques are based on direct staining of samples with fluorescein-labeled monoclonal antibodies (DFA). This last technique uses species-specific antibodies directed mainly against the antigen major outer membrane protein (MOMP) and to a lesser extent against the LPS. The main advantages of the DFA techniques are its speed (30 min) and specificity close to 100%, the sensitivity is 85-90%, compared to the culture and does not require specific means of transport. Among its drawbacks is the subjective interpretation and requires experienced staff, low reproducibility and the volume of samples should not be high.

Cell Culture

Until the end of the 20th century, cell culture has been the reference standard against which all other tests have been compared. But mainly due to the appearance of new diagnostic methods easier to implement, fast and sensitive, cell culture has been relegated to reference laboratories. Established cell lines for isolation of *C. trachomatis* include McCoy, HeLa 29.²¹ The specimens for culture must be collected using special devices and transport media. Sensitivity of culture may be impaired by inadequate specimen collection, storage and transport, toxic substances in clinical specimens and overgrowth of cell cultures by commensal bacteria and fungi. Cell culture is a very specific technique, however sensitivity is not very good 75-80%.^{21,32}

Neisseria gonorrhoeae (NG) infections

Gonorrhoea is the second most common bacterial sexually transmitted infection (STI) worldwide³³ although the prevalence of this infection varies among populations.³⁴ From a localized lesion, the microorganism can ascend to the upper genital tract to cause pelvic inflammatory disease, epididymo-orchitis or disseminate as bacteraemia. Due to this fact, an appropriate diagnosis and an effective treatment of this infection are important factors contributing to public health control and to prevent serious complications. However, the increase of resistance to recommended treatments for gonorrhoea may seriously affect to infection control.³⁵

Diagnosis of NG infections

Microscopy

NG could be visualized on microscopy of a stained genital tract smear in symptomatic patients. In men with urethral discharge, microscopy (x1000) using Gram for identification of diplococci within polymorphonuclear leukocytes has good sensitivity ($\geq 95\%$) and specificity ($\geq 99\%$) as a rapid diagnostic test.³⁶ However, in asymptomatic men, this technique has poor sensitivity ($\leq 55\%$), as well as in identifying endocervical or rectal infection ($\leq 55\%$ and $\leq 40\%$, respectively); in these circumstances, microscopy cannot be recommended as a test for ruling out infection. In addition, Gram stains of

endocervical, rectal, or pharyngeal specimens also are not recommended to detect infection due to poor specificity as well as low sensitivity.

Culture

Culture is the only diagnostic test that allows antimicrobial susceptibility testing, remaining important to detect and monitor antimicrobial resistance. Specimens should be obtained by using swabs other than those composed by wood and cotton because they may be inhibitory or toxic to NG. Some transport systems can maintain gonococcal viability for up to 48 h in ambient temperature.³⁷ Swabs should be inserted 2-3 cm in the male urethra or 1-2 cm into the endocervical canal followed by 2-3 rotations.

Specimens from sterile sites could be cultured on nonselective medium (e.g. chocolate agar), whereas those from nonsterile locations are cultured on a selective medium (e.g. Martin-Lewis, Thayer-Martin),³⁸ which contain antimicrobial agents that inhibit the growth of other bacteria and fungi. These media are incubated at 35 °C in an atmosphere supplemented with 5% CO₂ and examined during at least 48-72 h. Gram-negative diplococci and oxidase-positive colonies can be presumptively identified as NG. Further additional biochemical tests are needed in order to confirm the diagnosis.

Culture should be carry out for antimicrobial sensitivity study in patients with persisting infection or if treatment failure is suspected. Moreover, characterization of isolates by molecular typing may be a useful tool to predict antimicrobial resistance due to the fact that some types are associated to decreased susceptibility to several antibiotics.³⁹ The sensitivity of culture is high for genital samples but strongly depend on the specimen collection, transport, storage and isolation procedures.

Nucleic acid amplification tests (NAATs)

NAATs techniques are recommended for detection of infections caused by NG with and without symptoms. NAATs are more sensitive than culture, they can be used on a wider range of specimen types, and specimen quality, transportation and storage are less strict.⁴⁰⁻⁴³ NAATs are the sample of choice for testing asymptomatic patients.⁴⁰ These techniques show similar sensitivity in urine and urethral specimens from men,⁴³ and also similar sensitivity in endocervical specimens taken from physicians and those self-taken from the patients.⁴⁴ However, in women, urine samples have lower sensitivity than genital swabs for testing.⁴⁵ Moreover, NAATs are significantly more sensitive than culture for the detection of NG in pharyngeal and rectal samples,^{46,47} being the tests of choice for screening for these kind of infections. However, these techniques are not approved for testing specimens from these locations. A summary of the commercially available and FDA (Food and Drug Administration)-cleared NAAT assay platforms for the detection of NG in the United States can be

found in the current American guide for the diagnosis of these infections.³²

Treponema pallidum (syphilis) infections

Syphilis develops in stages, and symptoms vary with each stage (primary, secondary, latent, and late or tertiary syphilis, including neurosyphilis and cardiovascular syphilis). But the stages may overlap, and symptoms do not always occur in the same order. Alternatively, patients may be completely asymptomatic and only identified on routine screening. The choice of method for diagnosing syphilis depends on the stage of disease and the clinical presentation.

Direct detection methods

Dark field microscopy (DFM) and direct fluorescent antibody staining for *T. pallidum* (DFA-TP) have been used in clinical laboratories for decades to visualization of the spirochete in lesion exudate from patients with primary and secondary syphilis. However, these methods are not available in all laboratories, in addition to needing experienced personnel.

Nucleic acid amplification tests, such as the polymerase chain reaction (PCR), have not been used routinely for syphilis diagnosis since a commercial test is not available or internationally approved.⁴⁸ However, PCR tests for syphilis can be performed for the diagnosis of neurosyphilis, particularly among individuals infected with HIV.^{49,50} It is considered that CSF PCR has little value for diagnosis of neurosyphilis due to its low sensitivity and specificity. Its carrying out in blood is not recommended existence of inhibitory substances. To do this test can be used fresh samples or frozen. There are commercial formats, validated for all types of samples, so that it is essential to use the controls of corresponding validation.

Serological assays

Serological testing is the most common method for syphilis screening, diagnosis, and follow-up of treatment.⁵¹ Serological tests for syphilis can be divided into two types: nontreponemal test (NTTs) and treponemal test (TTs). Both tests are used to confirm the infection and determine whether the disease is active.

NTTs detect IgM and IgG antibodies to lipoidal antigens released from injured host cells.^{48,52} NTTs include Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and toluidine red unheated serum test (TRUST). Non treponemal antibodies become positive 10–15 days after the onset of the primary lesion. NTTs lack sensitivity in primary and tertiary syphilis and its use as a screening test presents problems. Without treatment, titres increase at 1–2 years after infection and can gradually decline spontaneously and, in some patients, become non-reactive. After treatment, titres generally decline and in most immunocompetent individuals become non-reactive within 6 months.

However, up to 20% of individuals infected and correctly treated show persistently reactive NTTs low titre results.^{53,54} False-positive results with this test can occur during pregnancy, in patients with rheumatological diseases, chronic infections (HIV, mycobacterial diseases) and parenteral drug users.

TTs use native or recombinant *T. pallidum* antigens to detect specific antibodies to treponemal components. These tests include the fluorescent treponemal antibody absorption assay (FTA-ABS), the *T. pallidum* particle agglutination assay (TPPA), enzyme-linked immunoassays (EIAs), chemiluminescence immunoassays (CIAs), and immunochromatographic assays (ICs). Specific antibodies are the first to appear (6–14 days after the primary chancre appears) and persist throughout life; TTs cannot be used to distinguish an active from a past or previously treated infection and, therefore, are not helpful for evaluation the effectiveness of antibiotic treatment.

FTA-ABS is considered the *gold standard* in many middle-income and low-income countries but it has drawbacks like time consuming, expensive and difficult to read. This assay can be used in cerebrospinal fluid (CSF). Sensitivity of TTs varies 82–100% depending on disease stage; specificity is 99%.

In recent years, rapid and inexpensive serologic tests for syphilis have been developed. Rapid syphilis tests are ICs assays that use a whole blood sample, require no equipment and minimal training and give a result in few minutes with a sensitivity of 86%.⁵⁵ Most tests use treponemal antigens but one IC test has been developed that enables the simultaneous detection of nontreponemal and treponemal antibodies in a single point of care device,^{56–58} and it can distinguish between new and previously treated infections. The overall performance for diagnosis of active infection is 88.3% (range 87.1–89.4%).^{56,58}

Interpretation of reactive tests

Screening using an initial automated treponemal test (EIA or CLIA) is done by many laboratories, especially those with high sample volume. A negative treponemal test likely indicates the absence of syphilis and generally no further testing is required. However, recent infection cannot be ruled out and repeat testing should be considered in patients who have had a recent risk exposure.

Reactive samples must be tested by a nontreponemal test to determine if disease is active. A positive titer with a VDRL or RPR indicates active syphilis and follow-up serologic testing is performed to monitor treatment response. When NTTs is not reactive in patients who report no history of syphilis treatment, it could be very early syphilis, longstanding latent syphilis, or a biological false positive result. Second different TTs should be performed. Patients with positive second TTs are candidates for treatment if they have not been previously treated.

Special situations

Neurosyphilis

Examination of CSF in a patient with neurologic signs and symptoms must include total protein, number of mononuclear cells and serologic test. CSF laboratory abnormalities (pleocytosis and an increased protein concentration) are common in persons with neurosyphilis. CSF-VDRL is highly specific but insensitive (a positive CSF VDRL test is observed in only about 1:3 cases of neurosyphilis). Rapid plasma reagin testing on CSF is not recommended.⁴⁸

In a person with neurologic signs or symptoms, a reactive CSF-VDRL (in the absence of blood contamination) is considered diagnostic of neurosyphilis. When CSF-VDRL is negative despite the presence of clinical signs of neurosyphilis, and abnormal CSF cell count and/or protein, neurosyphilis should be considered.

The CSF FTA-ABS test is less specific for neurosyphilis than the CSF-VDRL but is highly sensitive. In patients with suspicion of neurosyphilis but a negative CSF VDRL, a CSF FTA-ABS test can be used to rule out neurosyphilis.⁴⁸

Congenital syphilis

Because maternal nontreponemal and treponemal IgG antibodies can be transferred from mother to child, treponemal testing of infant serum is not recommended. A fourfold increase or more of the titre of a NTTs in the child's as opposed to the mother's serum (both obtained simultaneously at birth, is highly suggestive of congenital syphilis but its absence does not exclude a diagnosis.⁵² Children from mother with a positive treponemal test for syphilis and any evidence of congenital syphilis on physical examination, or long bone x-ray suggestive, or reactive CSF VDRL assay, or elevated cerebrospinal fluid cell count or protein (without other cause) can support a diagnosis of congenital syphilis.⁵²

Infections caused by *Trichomonas vaginalis* (TV)

TV is the most prevalent non-viral STI worldwide. These infections represent the most common curable STI in young, sexually active men and women. In women, trichomoniasis has been associated with poor reproductive health outcomes like low birth weight and premature birth.⁵⁹ Thus, early detection and treatment of TV infections are strongly recommended for symptomatic women and men. For asymptomatic patients, detection is only recommended for HIV-positive women.¹⁷

Diagnosis of TV infections

Conventional methods: microscopy and culture

Diagnosis of trichomoniasis is commonly based on the microscopy examination of wet preparations of vaginal and urethral discharges, prostatic secretions, and urine sedi-

ments. Specimens should be mixed with a drop of physiologic saline (but never refrigerated) and examined microscopically within 1 h under low power (magnification x100), with reduced illumination. The presence of actively motile trichomonas is diagnostic of the infection.⁶⁰ Polymorphonuclear cells are often present in these preparations. However, although this technique is rapid and inexpensive the sensitivity is between 50-70% and may be less in asymptomatic women. The most important factor affecting the sensitivity of wet mount testing is the time between collection and examination of the specimen.

Culture of genital fluids has been considered the gold standard for diagnosis, although it requires 18-24 h of incubation. The sensitivity of the culture is greater than 80% compared with the wet mount. Amies gel agar transport medium might maintain the viability for culture of TV on swabs held at room temperature for 24 ± 6 h before inoculation of specimen into a culture pouch. However, the best practice is that specimens be collected properly and inoculated immediately into the appropriate medium, such as modified Diamond's, Trichosel, or Holander's medium. Culture systems or systems that allow direct inoculation, transport, culture, and microscopic examination are commercially available.⁶¹

Rapid detection tests: point-of-care testing

Several antigen detection methods have been developed, being the main advantage that they are rapid and easy to perform. A latex agglutination test have demonstrated an excellent sensitivity.⁶² An immunochromatographic capillary flow assay is commercially available for qualitative detection of TV antigens from vaginal swabs.^{63,64} The OSOM *Trichomonas* Rapid Test Kit is a dipstick assay providing results in 10 min. This test has demonstrated good sensitivity and specificity compared with other diagnostic methods.⁶⁵ A rapid test has been developed for TV detection. This assay uses novel electrochemical end-point detection using a multicopy region of the TV genome as the assay target. The sensitivity and specificity achieved using this assay is comparable with that achieved for existing nucleic acid amplification test (NAATs).⁶⁶

The Affirm VP111 is a nucleic acid hybridisation test that uses synthetic nucleic acid capture probes and colour development detection probes; compared with NAATs, this technique had a sensitivity of 46%.⁶⁷

The AmpliVue assay uses isothermal helicase-dependent amplification (HDA) and targets a conserved repeat DNA sequence of TV.⁶⁸ This technique has been recently approved by the FDA for vaginal swabs. On the other hand, Solana *Trichomonas* assay is an in vitro qualitative NAAT for the detection of TV also using the HDA technology and the Solana instrument.⁶⁹ It was recently FD approved. Compared with a NAAT assay, the sensitivity/specificity was 89.7%/99.0% for swabs and 100%/98.9% for urines.

Finally, the GeneXpert TV assay has been approved by the Food Drug Administration (FDA) for use with male urine.

NAATs techniques

These techniques have now become commercially available for the diagnosis of TV in women and have replaced culture as the gold standard test due to their excellent sensitivity and specificity. Genital and urine samples are acceptable specimens.⁷⁰ NAATs have not been approved for men by the FDA, but these techniques have shown high sensitivity and specificity for this population. Among women, NAATs may detect a prevalence of threefold to fivefold higher than wet mount microscopy.⁷¹

Currently, there are two robotic FDA-approved NAAT platforms for the detection of TV in women: these include the Aptima TV assay (Hologic Gen-Probe)⁷⁰ and the BD ProbeTec Qx assay on the BD Viper system (Becton Dickinson).⁷²

Studies using the *Trichomonas* Aptima Combo 2 assay have shown a superior performance compared to other methods. This assay is approved for the detection of TV infections from a wide variety of specimens such as vaginal or endocervical samples, ThinPrep liquid-based cytology samples, and urine specimens.

On the other hand, the BD TV Qx uses female vaginal or endocervical swabs as well as urine and liquid-based cytology. This technique has demonstrated an excellent sensitivity and specificity, and the time of detection is less than 5 h.

Another assay available outside the US is the Seeplex STD 6 ACE detection system (Seegene).⁷³ This assay is a multiplex PCR targeting unique genes of the specific pathogen.

Finally, the BD MAXTM System provides an assay suitable for use with female urine and vaginal or endocervical swab samples. Male urine has not yet been evaluated for TV. This assay has a sensitivity $\geq 91.5\%$ and specificity $\geq 98.6\%$.⁷⁴

Human papillomavirus (HPV) infections

HPV cause the most frequent STI worldwide. In Spain, the prevalence of HPV infection in sexually active women is around 14%, although this prevalence may vary depending on the age group and the associated risk factors.⁷⁵ More than 100 HPV genotypes have been identified and it is estimated that 40 of them are located in the anal and genital region. Non-oncogenic genotypes (low-risk genotypes), mainly 6 and 11, might cause benign manifestations such as condylomas or genital warts. On the other hand, oncogenic genotypes (high-risk genotypes and probable/possible high risk genotypes) have been associated with the etiopathogenesis of the invasive cervical cancer.^{76,77} This type of cancer affects nearly 500,000 women worldwide every year with a mortality of more than 270,000 persons.⁷⁸

Cytology-based programmes have been the main approaches for screening, but these are not often available in most of the low/middle-income countries.⁷⁹ The WHO 2014 cervical cancer screening guidelines recommend that screening should be carried out at least once between the ages of 39 and 49 years, and this screening could be extended to women younger than 30 years if there is evidence of high risk for high-grade cervical intraepithelial

neoplasia. Testing for high-risk HPV genotypes has been incorporated into the screening and management algorithms elaborated by several scientific groups as well as for the FDA. HPV testing is recommended in patients over the age of 30 years-old who demonstrate an initial non-type specific high-risk HPV-positive result along with a negative cervical cytology result, and as triage in patients with undetermined cervical cytology results (ASCUS, atypical squamous cell of undetermined significance). The primary methods for HPV diagnosis have been cytology and histology. However, the detection of HPV is facilitated by recent advances in molecular biology to detect HPV DNA sequences in clinical specimens such as hybrid capture and polymerase chain reaction (PCR).

Diagnosis of HPV infections

The adequate samples for HPV detection are both endocervical brushed specimens (cervical exfoliated cells) or endocervical biopsies collected in liquid medium.⁸⁰ The endocervical brush should be introduced in the 2/3 of the endocervical canal followed by 4-5 rotations, and cervical biopsies should be frozen as soon as possible. Residual material from diagnostic formalin-fixed paraffin embedded blocks may also be used for HPV testing. On the other hand, urine samples have shown a lower sensitivity, so it has been not recommended for HPV screening. Also, samples of external genitalia, perineum, anus and/or oropharyngeal sites should be taken, both in women and men, if these locations are affected.

Conventional and monolayer cytology

The primary method for the detection of HPV is still the Papanicolaou stained smear. The Pap smear screening test for cervical cancer was introduced by George Papanicolaou in 1941⁸¹ and it has been associated with a sustained reduction in cervical cancer incidence and mortality rates.⁸² However, the effectiveness of this method has never been demonstrated in a randomized trial. The Pap test aims to identify abnormal cells obtained from the transformation zone, the junction of the ecto and endocervix, where cervical dysplasia and cancers arise. However, the Pap smear procedure has some limitations such as that inadequate samples constitute about 8% and false-negative rates close to 30% have been reported.

Thin layer or liquid-based cytology has now been widely implemented worldwide and has theoretical advantages over conventional cytology, mainly reducing the number of false-negative results. However, systematic reviews comparing conventional and liquid-based cytology have not consistently shown that liquid-based cytology detects significant cancer precursors more effectively than conventional cytology.^{83,84}

Histopathology

Patients who have abnormal Pap smears but no evidence of cervical lesions could be evaluated by colposcopy and further biopsy. The colposcopy can detect both low-grade and high-grade dysplasia but does not detect microinvasive lesions. Stains resulting after biopsy could be used to detect antigens or HPV DNA.

HPV nucleic acid detection

Commercially techniques for molecular HPV detection

In the market, there is currently more than 125 techniques for HPV detection. These techniques can be differentiated in four types:

- DNA detection techniques: the HPV DNA is detected from the capsid region as well as from the E6 oncogen.
- RNA detection techniques: the RNAm is detected from the HPV E6/7 oncogenes.
- *in situ* hybridization techniques: they have low sensitivity and specificity.
- Serological techniques: only used for epidemiological purposes and vaccinal efficacy.

According to the technology used, the main commercially available systems may be classified as follows:

- 1 Signal amplification methods: capture of RNA-DNA hybrids and Chemical Invader.
- 2 Target DNA amplification methods: PCR, real-time PCR, multiplex PCR, transcription mediated amplification (TMA), dual priming oligonucleotide (DPO) and Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Validation and FDA-approval

In 2009, an international expert committee proposed that any technology must be as accurate as the techniques used at this moment as the gold standard (GP5+/GP6+PCR and hybrid capture)⁸⁵ in order to be used in the primary screening of cervix cancer in women. This committee introduced some criteria based on both clinical sensitivity and specificity more than 0,90 and 0,98, respectively.

On the other hand, the VALGENT protocol is an international network for the validation of genotyping HPV assays. Moreover, the FDA approval is achieved when a method establish their sensitivity and specificity by prospective studies performed in three or more sites.

HPV detection techniques comparison

Abbott Real Time HR-HPV and BD Onclarity HPV are two DNA amplification techniques by RT-PCR fully automated. Abbott technology allows the process of the primary tube and reports genotypes 16/18 and others non 16/18 differently. This method is mainly indicated for laboratories with

high workload. BD Onclarity use SDA (Strand Displacement Amplification) technology and amplify the E6/7 region.

Anyplex II HPV HR (Seegene) is based on multiplex RT-PCR with DPO and TOCE technology. This method allows the genotyping of 14 genotypes in the same reaction and their relative quantification.

The Xpert HPV genotyping system (Cepheid) is the fastest test (1h). With this method, we may obtain the genotyping of HPV-16 and other 5 group of high-risk genotypes. Due to their low rate of contamination, it is recommended for laboratories low workload.

Other techniques clinically validated are the virus detection by MALDI-TOF MS and by reverse-hybridization with arrays in microspheres (Luminex).

Linear Array HPV Genotyping Kit (Roche Diagnostics) detects 37 genotypes, very useful for studies of vaccine impact or for epidemiological purposes.

Four techniques are FDA-approved for cytological screening of ASCUS or for screening with cytology and HPV at the same time: hybrid capture (Qiagen), Cervista (Hologic), Cobas 4800 HPV (Roche Diagnostics) and APTIMA (Hologic). Only the Cobas HPV test is approved by FDA for population screening based on HPV detection.

Hybrid Capture 2 High-Risk HPV DNA (Digene)

It was the first method approved by the FDA (March 2003) for the detection of HPV oncogenic genotypes. It is a liquid/solid phase signal amplification method based on hybridization in solution of long synthetic RNA probes complementary to the genomic sequence of 13 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 5 low-risk (6, 11, 42, 43, and 44) HPV types. The hybrids are detected by some reactions that generate a luminescent signal that can be detected by chemiluminescence. The main limitation of this assay is that does not discriminate the genotype and the cross-reactivity that may lead to false-positive results.⁸⁶

Cervista HPV HR (Hologic)

It was approved by the FDA in 2009. This assay is based on *Invader* technology consisting of concurrent two-part isothermal reactions. The main reaction detects the presence of specific viral DNA sequences, while the second one generates fluorescence. The presence of any of the 14 high-risk HPV genotypes could be detected (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), but not in an individualized manner. However, Cervista HPV 16/18 identify HPV 16 and 18 individually.

Cobas HPV Test (Roche Diagnostics)

The Cobas 4800 system is an automated method that uses the primary sample obtained for the liquid-based cytology. The results appear differentiated in four channels: HPV 16, HPV 18, high-risk HPV non 16/18 (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and β -globin (internal control). The main advantages are the high sensitivity, reproducibility, and high-degree of automation.

Aptima HPV Assay (Hologic)

It is an assay that identifies the presence of 14 high-risk genotypes by the identification of the viral RNA from the E6/7 oncogenes. The presence of transcripts of the HPV oncogenes is a more accurate and specific marker of cell infection or transformation by high-risk HPV. This method is useful in differentiating between episomal and integrated HPV oncogenic transcripts, such as in cervical cancer. This technique consists of three steps: capture, amplification by TMA system and detection by hybridization.⁸⁷ However, the main problem with this technique is that RNA is much more labile than DNA and less available in most biological specimens.

Microarrays (DNA chips)

Recent developments in combining molecular probes with silicon-based chips may lead to quick and relatively inexpensive diagnostics. This technology requires the use of silicon chips; the surface of the chip is covered with a fine layer of gold, and molecular probes are attached to the surface of the chip. Each of the molecular probes differs in the DNA target they are designed to hybridize. If binding is detected, the sample would be considered positive for HPV.

Serological assays

The majority of studies employed enzyme immunoassays, but the main problem with the use of the serology is standardization and the establishment of an international standard assigning one unit measure or international unit. Studies have demonstrated that about half of people exposed to HPV never developed measurable titers of antibodies.⁸⁸

Utility of P16^{INK4a}

The over-expression of this protein has been proposed as a tissue marker for high-risk HPV infection. Positivity of this protein increases with lesion severity, and a high percentage of HSIL cytology specimens are positive. The sensitivity of P16^{INK4a} assays to detect CIN3 is similar to HPV DNA assays, but the specificity needs to be improved. Its role as a molecular prognosis factor is still an issue pending assessment.⁸⁹

Herpes simplex virus genital infection

Genital herpes is a common sexually transmitted disease (STD). It is caused by herpes simplex virus type 2 (HSV-2) or herpes simplex virus type 1 (HSV-1). Most people who have HSV-1 or HSV-2 do not have symptoms.

The laboratory diagnosis of genital herpes is recommended in confirmation of clinically suspected genital herpes or differential diagnosis with other ulcerative STIs or genital ulcerative dermatoses, and in extra-genital complications of genital herpes.

For active lesions, collection of vesicular fluid or exudate from small vesicles with cotton or Dacron swab is the method of choice for collecting samples. The laboratory methods for direct herpes diagnosis include viral culture, antigen detection and DNA detection based on nucleic acid amplification by PCR.

Viral isolation

Tube culture isolation is the traditional gold standard for HSV detection. HSV grows readily in a wide variety of cell lines but the cell lines most frequently used for HSV culture are fibroblasts, MRC-5 and Vero cells. While the test has 100% specificity for HSV-1 or HSV-2, the sensitivity depends on the stage of the lesion at the time of specimen collection. The sensitivity also varies from 75% for first episodes to 50% for recurrences.^{90,91} Shell vial culture can reduce viral isolation times from one to seven days to 16 to 48 h. However, although these methods are rapid and specific, they are slightly less sensitive than traditional tube cultures and are more expensive.⁹²

Antigen detection

Viral antigen can be detected by direct immunofluorescence assay (DFA) or enzyme immunoassay (EIA). The IF assay is a satisfactory and rapid (<4 h) method for diagnostic (sensitivity 80% and specificity 90%) but requires samples from fresh vesicles.⁹³

Virus detection by molecular biology

PCR assays or other NAATs are the most sensitive test currently available to detect HSV in clinical samples. Real-time PCR is faster, less labor-intensive than traditional PCR and in the presence of active lesions, PCR is the preferred test, with sensitivity and specificity greater than 95%.^{94,95}

Serological diagnosis

Serologic testing may be useful in patients with recurrent genital symptoms or atypical symptoms and negative herpes simplex virus PCR. In addition, serological assay is useful knowing infection status in partner with genital herpes. If genital lesions are present, type-specific serology and direct virus testing can help to establish if the episode is a reactivation or a new HSV infection.

HSV IgM testing has limited availability in routine diagnostic settings and cannot be recommended in routine clinical practice. Type-specific HSV IgG antibodies are negative in early stages of herpes disease, and become detectable two weeks to three months after the onset of symptoms and persist indefinitely. Type-specific HSV glycoprotein G based ELISA tests are recommended for serological diagnosis. Primary HSV infections can be documented by seroconversion with paired sera. The sensitivities of these IgG tests for the detection of HSV-2 antibody vary from 80 – 98%, and the specificities of these assays are $\geq 96\%$.⁹³ False-negative results can occur in period window of two weeks to three months after HSV exposure.

Mycoplasma genitalium infection

Since the availability of molecular assays, *M. genitalium* has been associated with many adverse disease outcomes, such as non gonococcal urethritis in men and many adverse reproductive sequelae in women like cervicitis, endometritis,

preterm birth, spontaneous abortion and pelvic inflammatory disease.^{96,97} Other studies have reported promotion of HIV acquisition and shedding by antecedent *M. genitalium* infection.⁹⁸ However, *Mycoplasma hominis*, *Ureaplasma urealyticum* (previously *U. urealyticum* biovar 2) and *U. parvum* (earlier *U. urealyticum* biovar 1) are frequently found in the human urogenital tract in both healthy individuals and symptomatic patients.⁹⁹

A number of commercially produced *M. genitalium* DNA amplification modalities have been described. *M. genitalium* specific oligonucleotide primers have been incorporated into single or multiplex PCR assay for six ITSs (e.g. Seeplex STD6, Seegene) in urogenital specimens or vaginal and urine sample by PCR microarray (STDetect chip, Lab Genomics). Other attempts to detect *M. genitalium* DNA come in the context of assays designed for to detection of *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. urealyticum* from male first void urine sample together with other pathogens (e.g. FilmArray STI panel, BioFire Diagnostic).⁹⁹

There are also other assays that have CE marking (Biorad DX CT/NG/MG, Biorad) (Hyplex STD Mycoplasma test, Amplex Bioystems), the latter has shown a sensitivity and specificity of 87% and 96% for detection of *M. genitalium*.¹⁰⁰

There have been several published research assays that can detect macrolide resistance in samples known to be positive for *M. genitalium*, using detection of 23S RNA gene mutations that are associated with resistance by PCR and melt curve analysis.¹⁰¹ Plex Zyme and PlexPrime recently developed in 23S assay a multiplex qPCR assay for detection of *M. genitalium* and the 5 mutations associated with macrolide resistance, the assay was evaluated in 400 samples from 254 consecutively infected participants, 56% showed a macrolide resistance mutation and its sensitivity and specificity were 99.1% and 98.5% for *M. genitalium* detection and 97.4% and 100% for macrolide resistance.⁹⁹

HIV infection

At present in our country surveillance data suggest a stabilization or decrease in HIV incidence in the face of apparent increases in the numbers of persons tested among key risk groups. The reasons for these improving trends are not yet clear, but in order to be sustained, we must continue to refine systems for HIV testing and linking persons to care and prevention resources, as appropriate.¹⁰²

HIV testing is often prompted by a defined exposure, such as a needlestick injury, condom failure, or condomless sex.

Following an exposure that leads to infection, there is a variable amount of time called the eclipse period in which no existing diagnostic test is capable of detecting HIV. HIV RNA is the first reliable marker of infection; 50% of infected individuals have detectable plasma RNA within 12 days and levels peak between 20–30 days. Beginning around day 15, the HIV-1 capsid protein p24 reaches detectable levels in the plasma. Antigenemia with p24 continues to rise through days 25–30, at which point early anti-HIV antibodies are able to complex with circulating p24; by day 50, antigen is often cleared from the bloodstream entirely. This short-lived detectability of p24 is therefore helpful in determining recency of infect, but also makes its utility in diagnosis time limited.^{103–105}

All HIV diagnostic testing is guided by a common principle: screen with a highly sensitive initial test and confirm reactive results with a different test that is both sensitive and highly specific. This can be accomplished using two POC tests, two laboratory-based assays, or combinations thereof; all of these strategies have been studied extensively. Since the US Food and Drug Administration (FDA) approved the first HIV diagnostic test in 1985, four additional “generations” of antibody tests for HIV have been developed; each improves incrementally on its predecessor(s) in terms of performance and shortening of the window period.^{106,107}

IgM/IgG sensitive (formerly third generation) tests shorten the window period to the earliest threshold of IgM detection – a median of 23 days after infection.¹⁰³

Antigen/antibody (Ag/Ab) combination (formerly fourth generation) tests pair an IgM/IgG sensitive antibody test with simultaneous, separate p24 antigen detection. Some of these p24/IgM/IgG sensitive tests report a reactive result if any element is detected, while others yield separate results for p24, anti-HIV-1 antibodies, and anti-HIV-2 antibodies. Detecting p24 shortens the median window period down to just 18 days following infection.

In contrast to complex, automated laboratory-based platforms, POC tests rely on one of two methods: lateral flow, in which the specimen is drawn through an antigen-impregnated strip by capillary action; or flow-through, in which the patient’s specimen and reagents are sequentially applied to a membrane embedded with HIV antigens. Laboratory-based serum or plasma assays generally offer higher sensitivities, but require venipuncture, larger specimen volumes, processing, and skilled technicians. POC tests are attractive alternatives for many applications, but performance differs substantially depending on the specimen type; tests using oral transudate are significantly less sensitive than those using whole blood, and tests using whole blood are less sensitive than those using serum or plasma.^{108,109}

In summary, the laboratory test is one of a number of tools in the diagnosis of the patient with STIs, and so the clinician should always be aware of the tests the laboratory offers and the results of any tests should be interpreted in the clinical context. It is likely that POCT based on molecular biology will be more common in the future to allow rapid diagnosis, but should be used with laboratory support.

Conflict of interest

None.

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